Developing Southeastern adapted White Lupin (Lupinus albus) varieties as a multi-purpose cover crop and a grain legume

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Grant: 2024 Southern Graduate Student Grant

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Amount Requested: $21,926

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Description for search results if funded: This project aims to evaluate the potential to develop, by plant breeding, a new and versatile cool-season legume, the White Lupin, to fulfill multiple needs of southeastern cropping systems, such as another cover crop option and winter-grown protein source.

Project start date: September 01, 2024

Project end date: August 31, 2026

Basic Information

Major Professor and Graduate Student Experience and Roles

Dr. Marnin Wolfe will be the Principal Investigator and will play a supervisory role in the project. Dr. Marnin Wolfe is an Assistant Professor of Quantitative Genetics with about 11 years of experience in plant breeding. Dr. Wolfe's Lab is focused on using breeding and genetics to develop better cover crops and forages at Auburn and to benefit the sustainability of cropping systems in the Southeast more generally. The lab focuses mainly on legumes like the White Lupin we propose to evaluate as part of this proposal.

Oluwaseye Gideon Oyebode has an MSc in Plant Breeding and Genetics. He is in his second year of a PhD program at the Department of Crops, Soils, and Environmental Sciences, Auburn University, supervised by Dr. Marnin Wolfe. Gideon has nine years of practical Plant breeding experience; he previously worked with the NextGen cassava breeding program and, recently, as a Research Associate and breeding operations manager for the Cowpea breeding programs of the International Institute of Tropical Agriculture (IITA), Nigeria. Oluwaseye will lead the project execution, trial establishment, data collection and analysis, and report writing under the supervision of Dr Marnin Wolfe.

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State

Alabama

Proposal

Project Abstract

Winter rainfall on bare agricultural fields leads to soil erosion and nutrient runoff, resulting in eutrophication and degraded quality in nearly 40% of Southeastern waters. Cover crops are those planted between cash crop seasons to protect and benefit the soil. Cover cropping is on the rise, and so is the demand for new and improved species and cultivars bred for the purpose. Concurrently, rising demand for plant-based proteins and fluctuations in global soybean prices highlight the need for alternative protein sources. White Lupin (WL) is a versatile cool-season legume that can fulfill multiple needs of southeastern cropping systems. WL stands out among winter legume species; it is among the top N-fixing legumes and has a unique ability to obtain immobilized phosphorus thanks to specialized root structures. Currently, no commercially adapted WL varieties exist for the Southeast US. Fortunately, significant genetic diversity is available to us to develop one rapidly.

This project aims to evaluate the potential to develop, by plant breeding, Southern-adapted WL to fill both economic and environmental gaps. Our project will leverage Auburn University's unique breeding materials in combination with the USDA collection. In a two-year trial, we will target traits related to biological nitrogen fixation, phosphorus acquisition, root structure, grain yield, and forage/nutritional quality. Lastly, we will use genotyping to explore WL's genomic diversity and the possibility to use DNA-markers to develop new varieties rapidly. Ultimately, this will help identify the most cost- and time-efficient methods to develop WL cultivars for sustainable Southeast cropping systems.

Statement of Problem, Rational and Justification

The purpose of this project is to evaluate the potential to develop, by plant breeding, a new and versatile cool-season legume, the White Lupin, to fulfill multiple needs of southeastern cropping systems.

Winter rainfall on bare agricultural fields leads to soil erosion and nutrient runoff, resulting in eutrophication and degraded water quality in nearly 40% of the region's waters (McCarty et al., 2008). Cover crops (CC) are plantings made between cash-crop seasons to benefit the soil rather than providing direct economic benefit. CC are, therefore, an integral component of sustainable agriculture, offering soil health benefits such as erosion and nutrient runoff control and enhanced biodiversity. The utilization of CC is increasing, yet challenges remain in realizing their full economic potential. For instance, 33% of farmers are yet to realize significant savings on fertilizer costs from using CC (National cover crop survey, CTIC & SARE 2023). There is substantial opportunity for innovation in the development of better CC, especially nitrogen-fixing legumes, which can address both economic and environmental gaps.

White Lupin (WL) (Figure 1) is a promising solution to these challenges. WL stands out among winter legume species with its superior ability to fix nitrogen (Torbo and Bhardwaj 2023), which meets or exceeds that of other CC legumes, like Crimson Clovers. WL also features a unique cluster root system not present in other legume CC, which produces carboxylates that mobilize soil-bound phosphorus (Pavinato et al. 2017), making both nitrogen and phosphorus available to subsequent cash crops (Kalembasa et al., 2020). Such capabilities can significantly reduce the reliance on chemical fertilizers, potentially lowering both agricultural costs and environmental impacts. WL’s deep roots stabilize the soil structure, reducing erosion and runoff, and contribute to building organic matter (Robson et al., 2002).

The Southeast also needs to diversify its plant protein sources (USDA, 2024; Torbo and Bhardwaj, 2023) as global protein demand is expected to increase by 20% between 2018 and 2025 (Torbo and Bhardwaj, 2023). Farmers need crops that not only thrive in their specific agricultural environments, but also complement existing crop rotations without competing for resources. WL fits this niche perfectly, presenting a viable complement to dominant crops like soybeans. WL contains 32% - 36.9% protein with concentrations of essential amino acids comparable with soybean (Gresta et al., 2023) and forage quality comparable to alfalfa (Torbo and Bhardwaj, 2023).

Currently, no commercially adapted WL varieties exist for the Southeast US. Fortunately, the USDA National Plant Germplasm System (NPGS) contains a collection of WL accessions and Auburn University has a collection from previous breeding efforts. In 2022-2023, we revived Auburn’s WL germplasm.

The first step in WL development involves characterizing the phenotypic and genotypic diversity of these untested WL germplasms. By genotyping and phenotyping, we can identify lines with valuable traits using genomics-assisted selection, and fill the variety gap. Adapting WL to the Southeast adds another CC and protein source to regional farmer’s options and, as such, will potentially contribute both ecosystem services and sustainable intensification of protein sources.

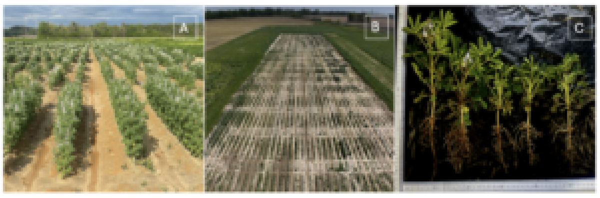

Figure 1: A: Section of our 2023-2024 trial at the flowering stage; B: Aerial view of the field; IC: Variation in root cluster and nodules among 5 white lupin accessions ten weeks after planting

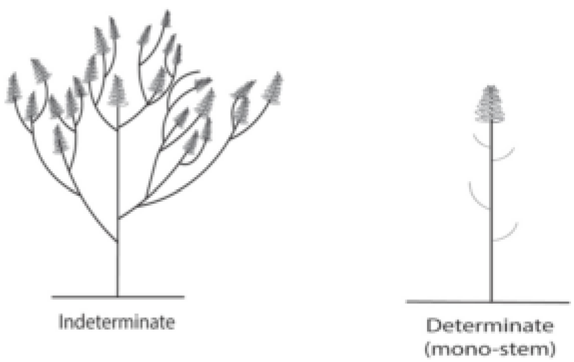

Figure 1 D: illustration of indeterminate and determinate plant types

Objectives

Objective 1: Assess WL diversity for genetic variability in traits related to cover crop and grain legume performance.

Objective 2: Explore the potential to improve N-fixation and P-acquisition by evaluation of root architecture and N-fixation ability.

Objective 3: Study the genetic diversity of WL and the prospect of using genomics to develop varieties rapidly.

Relevance to Sustainable Agriculture

Crop production intensification in the Southeast, fueled by mechanization and extensive use of herbicides and fertilizers, enhances yields but leads to serious environmental consequences like soil erosion, aquatic ecosystem pollution, and greenhouse gas emissions, which pose a significant threat to food security (Environmental Protection Agency, 2021).

Cover crops planted during the winter have been identified as one of the most cost-effective conservation methods for reducing nutrient runoff due to agricultural practices (McCarty et al., 2008). For instance, from a study of 40 farms, Brittany et al., 2021, reported that cover crops reduced monthly totals of tile $NO3^{-}$-N and TN loads by ~1.0–2.6 kg N ha^{-1} from January to June (winter and spring), coinciding with critical periods of nutrient loss from agroecosystems in the midwestern US and Sangchul et al., 2017 reported annual nitrate loads reduction by 48% when winter cover crops are planted.

We hypothesize that by genetically improving the ability of WL to perform these eco-physiological functions, we improve the ability of the crop to provide ecosystem services, which significantly improve the soil structure and reduce nutrient runoff leading to safer environment and healthier soils. With the upward trend in the use of cover crops continuing (National cover crop survey, 2023), improving the available cover crops themselves will contribute directly to the ecosystem services that they are able to offer. We propose that the development of novel cover crop cultivars by breeding is an underutilized avenue to enhancing sustainable agriculture.

We target WL in this project because of its multifold potential to contribute to sustainability. Our project will aim to boost N-fixation and immobilization of inorganic phosphorus by breeding and selection of WL with the best ability to provide those services. WL is the only winter legume cover crop that produces cluster roots, an adaptation to low soil phosphorus (P). Cluster roots exude large levels of P-solubilizing compounds, organic anions such as citrate and malate and phosphatases that mineralize organic P (Bayon et al., 2006; McCarty et al., 2008). The P in the cover crop biomass is protected from sorption in the soil or losses by erosion and leaching. After termination and during the decomposition of cover crop residues, P taken up by cover crops can become available to the succeeding crop (Bayon et al., 2006). Paviato et al. (2017) found that over a three-year study, white lupin recycled and made available more phosphorus (P) to subsequent crops compared to other cover crops such as hairy vetch, forage radish, ryegrass, black oat, and red clover. Furthermore, WL’s deep roots help to stabilize the soil structure, resulting in the reduction of erosion and runoff and contributing to the building of organic matter (Robson et al., 2002).

WL used as a winter cover crop produced significantly higher yields in corn and muskmelon when compared to hairy vetch, Australian winter pea, and a control plot that received 112 kg N/ha (Torbo and Bhardwaj 2023). Likewise, Anderson et al. (2022), in a 5-year study comparing biomass yields of five leguminous winter cover crops (Austrian winter pea, fava bean, narrow-leaf lupin, cahaba vetch, and crimson clover) and rye, reported that narrow-leaf lupin (a close relative to WL) produced the most biomass and contributed around 143 kg ha^{-1} N across years.

In addition to being the only winter legume species with specialized cluster roots for phosphorus acquisition, WL is a top nitrogen fixer, which occurs through a symbiotic relationship with soil bacteria known as rhizobia. These bacteria colonize the roots of the lupin plants, forming specialized structures called nodules. Biological nitrogen fixation (BNF) is the process that converts atmospheric nitrogen in the root nodules of WL into forms that plants can use for growth. This nitrogen is then trapped in plant tissues and eventually benefits the soil and subsequent crops when lupin plant residues decompose, contributing to soil fertility and reducing the need for synthetic nitrogen fertilizers. Hence, the use of legumes such as WL in cover cropping is an effective, low-cost method for improving soil and water quality because they decrease synthetic fertilizer inputs and reduce soil erosion and compaction, thereby ameliorating the negative effect resulting from excessive agricultural pollutants in the soil and water bodies.

Cultivating WL as a high-protein winter crop in Alabama and the Southeast USA can contribute significantly to the sustainable intensification of agriculture through various mechanisms. In a systematic review, Bryant et al. (2022) found that whole lupin consistently improved satiation, glycemic control, and blood pressure in most studies, indicating the potential of WL to improve human health. Also, diversifying crop rotations with WL can contribute to agricultural biodiversity. A diverse crop rotation can reduce pest and disease pressure, reducing reliance on pesticides (Bowels et al. 2020). Integrating legumes like WL into crop rotations can increase the diversity of plant species and associated organisms such as pollinators and beneficial insects. This is crucial for ecosystem stability and resilience and essential for sustainable agricultural practices.

The demand for plant-based proteins is growing, and WL can be processed into various food products, providing farmers with new market opportunities. Considering that WL requires less application of synthetic inputs with harmful effects on the ecosystem, its inclusion into the farming system and adoption as a complementary protein source will reduce the reliance on soybeans, leading to reduced use of fertilizers and pesticides. Economic analysis suggests that WL can offer competitive profit margins compared to popular winter crops like wheat, especially with increasing demand for sustainable and local protein sources (Gresta et al., 2023).

Diversity, Equity and Inclusion

Our research is designed to positively impact all communities across Alabama and the Southeastern US sustainably. The key outputs from this project are a list of best-performing genotypes for each of the evaluated traits and data to implement a modern white lupin breeding program. These outputs will initially guide our breeding program work, which is upstream of producers, but our results will ultimately be made available for public consumption through extension publications and field data conducted throughout the state. The products of our work will be white lupin varieties serving with superior performance for multiple purposes (cover crops, forages, and grain legumes). Downstream research will involve conducting on-farm evaluations, including those of underserved communities such as Black Belt Prairie.

Economically challenged farming communities in the Black Belt produce corn, cotton, soybeans and small grains under hot and humid climates and on clayey soils. In addition to being an alternative source of plant protein, WL will enable double cropping within a year without competing with the production of other cash crops, enhance soil quality and cleaner water, and serve as a winter revenue opportunity for farmers who face challenges in growing soybeans and other cash crops due to the high input requirements of pesticides, herbicides, and fertilizers. Farmers will have a double advantage: cash from harvest in the form of forage, hay, or grains and better soils, which will nurture sustainable intensification of agricultural systems in underserved communities.

Approach and Methods

Germplasm Collection and Seed Increase Trials So Far:

From 2022 to 2023, we acquired 227 accessions of WL from the USDA NPGS. We also had 214 breeding lines unique to Auburn, derived from previous breeding efforts here. This includes an advanced WL selection we call “AU22” that is tall, but lodging resistant, and is alkaloid free, making it suitable for human/animal consumption. Our first trial with WL was primarily used to increase seed as we started with limited amounts per accession. We used this first trial and our in-progress 2023-2024 trial to characterize the germplasm relative for two traits: plant type and alkaloid-status. WL plants vary in their plant architecture, with some showing determinate growth (DET) and others indeterminate (INDET). Some, but not all WL plants genotypes sequester a potentially toxic alkaloid that requires post-processing before safe consumption. From our first trial, 337 genotypes produced seed: 196 were DET-type and were mostly from AU, 141 were INDET (mostly from USDA/NPGS lines). The DET types had no alkaloids, which makes them good for feed/food crops. AU22 and 28 USDA lines are the only indeterminate plant types without alkaloids.

In 2023-2024 we are conducting a preliminary yield trial with two-row 15-foot-long plots and two-replications each of the 200 lines selected from 2022-2023. We selected to test all 141 INDET types and the best of the DET types. This choice was to maximize diversity of INDET plant types while introducing more alkaloid-free genetics in our current trials and provides variation in maturity timing.

Objective 1: Assess WL diversity for genetic variability in traits related to cover crop and grain legume performance.

Experimental Design:

To achieve Objectives 1 and 2, we will conduct a two-year field evaluation for the project: Year 1 (2024-2025) and Year 2 (2025-2026). We will use the seed increase and data generated during the 2022-2023 and 2023-2024 seasons to select 200 lines from the available 441 WL accessions. We will use two 36”-spaced rows per 15-foot-long plot. Given sufficient seed is produced this year, we will test at two locations with two replications each. Trials will be conducted primarily at the EV Smith Plant Breeding Unit (PBU) in Tallassee, AL, and at a second site to be determined.

Data Collection: This objective focuses on agronomic data. All plots in both years will be regularly assessed for maturity timing using a weekly census. Stand count, growth in vegetation height/volume, and health indices will be captured using a multispectral-equipped drone, flown every 2-3 weeks. We will take notes on plant architecture (INDET vs. DET), alkaloid status, and flower color. Before seed harvest, biomass will be sampled (see Objective 2 below) as a cover crop performance indicator. At seed maturity, plots will be harvested by Combine for total seed yield and 100-seed weight.

Objective 2. Explore the potential to improve N-fixation and P-acquisition by evaluation of root architecture and N-fixation ability.

At maturity, but before seed harvest, we will harvest the above and belowground biomass from four plants per plot. Biomass samples will be dried at 60oC for 7 days and weighed. All aboveground samples will be ground finely to pass a 1 mm sieve and homogenized.

Carbon and nitrogen isotope analysis:

Between 3-5 mg of tissue from the PBU location will be weighed into tin capsules and submitted to the UC Davis Isotope Analysis Lab for assessment of C and N isotope content. The relative content of δ15N:δ14N is indicative of the proportion of tissue N derived from atmospheric N and thus from biological fixation (Sanz-Saez et al. 2019, Kalembasa et al., 2022). Furthermore, the ratio of C:N is critical for understanding the value of plant biomass both as a mulch (cover crop quality) and forage.

Analysis of cluster roots by imaging:

In both years, root systems of the same plants sampled for biomass and isotope analysis in field trials will be dug up and carefully cleaned. Roots will be dried, weighed and imaged to assess properties of their architecture, namely to detect variation in cluster root formation. The roots will be imaged using equipment operated by the AU Crop Physiology Lab. The platform (Fig. 2) plus RhizVision Explorer software can analyze images of roots taken from multiple angles simultaneously, to measure various traits, including length, diameter, volume, and branching patterns. This functionality makes it a valuable tool for investigating the root cluster and architecture of white lupin.

Data analysis: For Objectives 1 and 2, a mixed model will be used to assess the genetic and environmental variance components, as well as genetic parameters such as the heritability of the traits. WL lines will be ranked based on trait performance to identify the best-performing lines for each trait. Multivariate analysis will be used to understand and summarize the genetic correlations among traits, as these correlations determine how breeding improvements can / will proceed under selection. Cluster analysis will be used to classify the WL lines into distinct groups based on the similarity of their trait profiles. This classification can identify lines with desirable combinations of traits that can be targeted for selection in breeding programs.

Objective 3: Study the genetic diversity of WL and the prospect of using genomics to develop varieties rapidly.

We will use low-pass DNA-sequencing to obtain genome-wide single nucleotide polymorphism (SNPs) genotypes for the entire panel of WL. Genotyping the WL panel will unlock several possibilities. Firstly, to our knowledge, this will be the first genomic examination of the diversity of WL germplasm in North America. We will quantify genetic diversity, assess population structure and estimate genetic relatedness between and within the germplasm collections (NPGS and AU). Second, we will conduct genome-wide association analysis (GWAS) to identify genetic variants associated with traits-of-interest. This will be informative about the genetic complexity of the traits and, thus, the selection strategies that will be most effective. Finally, we will assess the prospect to use genome-wide selection (GS) by testing genomic prediction accuracy using cross-validation.

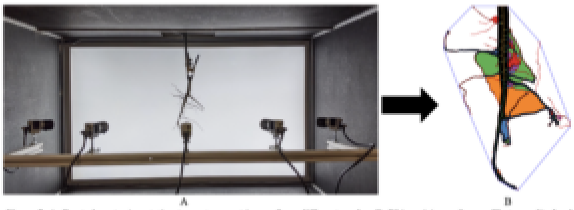

Figure 2: A. Root phenotyping station to capture root image from different angles. B. Rhizo vision software. Photo credit: Jagdeep Singh, Crop, Soil and Environmental Sciences, AU

Timetable

1. The experiment leading to achieving Objective 1 for Year 1 is ongoing and will be harvested in June 2024. Year 1 and Year 2 Field experiments will be established in the Fall of 2024 and 2025, respectively, and harvested in Spring 2025 (Year 1) and Spring 2026 (Year 2).
2. Data collection for Objective 1 will be conducted throughout both field seasons.
3. Collection of tissues for isotope and P analysis (linked to Objective 2) will occur in the Spring of each year when plants are approaching seed harvest.
4. Root imaging for Objective 2 will be conducted close to the harvesting of the trials in both Years 1 and 2.
5. A preliminary data analysis will be conducted at the completion of the Year 1 trial over the Summer of 2025, while a “Combined analysis” for Year 1 and 2 will be completed in the Summer of 2026.
6. Tissue collection for genotyping was done in 2022-2023 field season with supplementary tissue collected in 2023-2024. Genotyping to achieve Objective 3 will therefore be done in the Fall of Year 1 and thus data will be available to support analysis throughout the field component of the project. Most downstream analysis will be completed by September 2026 (this is to allow the use of 2-year phenotypic data).
7. Submission of the project report and publication of results will be in Fall of 2026.

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Budget

Budget Justification

Budget Total: $21,926

Category	Description	Amount
Fringe Benefits (Graduate Student)	PERSONNEL + FRINGE	$2,508
Material and Supplies	Materials and supplies for experiment	$500
Other Direct Costs	Genotyping and sequencing of white lupin germplasm to enable genomic study/selection	$11,025
Other Direct Costs	Carbon and Nitrogen isotope analysis	$4,400
Personnel (General Labor)	Undergraduate student labor	$500
Travel	Graduate student to attend one national conference to present results and findings	$1,000
	Total Direct costs: ***(includes subawards)***	**$19,933**
Indirect Costs	Indirect Costs	$1,993
	Total: $21,926	**$21,926**

Indirect costs: 9.9985%

Category	Details/Justification
Fringe Benefits (Graduate Student)	**PERSONNEL + FRINGE - $2,508** $2,417 are budgeted for Gideon Oyebode to receive for one-month (0.08 FTE, $29,000 salary) Graduate Research Assistantship to support his efforts leading the execution of the field trials, data collection and analysis of this project. Additionally, $92 are required for fringe benefits at a rate of 3.8%.
Material and Supplies	**Materials and supplies for experiment - $500** Total funds of $500 is requested for costs of materials and supplies associated with running the field trials and collecting the data described in the proposal narrative. Materials include: Bradyrhizobium lupine inoculum, seed tags, labels, coin envelopes, harvest bags, and field flags.
Other Direct Costs	**Genotyping and sequencing of white lupin germplasm to enable genomic study/selection - $11,025** Genotyping of White Lupin Germplasm: Funds of $11,025 are requested for genotyping of 441 White lupin lines at an estimated cost of $25/sample. Cost is for DNA extraction and sequencing/genotyping of lupin germplasm. This will enable the quantification of genetic diversity, relatedness and the implementation of genomics-assisted breeding as described under Objective 3

Other Direct Costs	**Carbon and Nitrogen isotope analysis - $4,400** Carbon and Nitrogen Isotope Analysis: Funds of $4,400 for Carbon and Nitrogen isotope analysis at a cost of $11/sample. This will allow the assessment of the variation in biological nitrogen fixation (Nitrogen isotopes) and water use efficiency (Carbon isotopes) performance among lupin germplasm, as described in Objective 2.
Personnel (General Labor)	**Undergraduate student labor - $500** $500 is requested for Undergraduate Student Support. Students will be hired at a rate of $11/hr or greater to perform seed preparation, harvesting, imaging, and field data collection.
Travel	**Graduate student to attend one national conference to present results and findings - $1,000** This covers the registration fee, travel, accommodation, and food for Graduate student to attend a conference (appropriate conference to be determined) to present results
Indirect Costs	**Indirect Costs - $1,993** Indirect Costs of $1,993 are calculated at 10% Total Direct Costs

Signature Page

Signature Page

Signature-Sheet Wolfe signed - 10May2024

SOUTHERN REGION SARE
2024 Graduate Student Grant
Proposal Signature Sheet

Project title: Developing Southeastern adapted White Lupin varieties as a multi-purpose cover crop and a grain legume.

Organization/Institution: Auburn University

Principal investigator: Marnin Wolfe

Total amount requested: $ 21,927

Organizational Administrative Representative: Steven Taylor, SVPRED

Signatures

Principal Investigator Signature   Date: 5/10/2024

Organizational Administrative Representative Signature   Date: 5/10/2024

NOTE: The signature page -and ONLY the signature page- may be received at the address below after the proposal deadline. Should your proposal be selected for funding, we must have your signed signature page on file in order to make the award.

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